Claims

1. A method of probing for a nucleic acid comprising: contacting a nucleic acid solution with an oligonucleotide probe labelled with an electrochemically active marker, providing conditions at which the probe is able to at least partially hybridise with any complementary target sequence which may be present in the nucleic acid solution, selectively degrading either hybridised, partially hybridised or unhybridised nucleic acid probe, and electrochemically determining information relating to the electrochemically active marker.

2. A method as claimed in claim 1 wherein the information relating to the marker is used to derive information concerning the presence or absence of at least one nucleic acid species.

3. A method as claimed in claim 1 or claim 2 wherein the electrochemical technique is used to quantify relative proportions of degraded and non-degraded probe.

4. A method as claimed in any one of claims 1 to 3 wherein nucleic acid probe that has failed to successfully hybridise is digested by an enzyme that has been chosen to selectively digest single stranded unhybridised nucleic acid.

5. A method as claimed in claim 4 wherein the enzyme is an endonuclease.

6. A method as claimed in claim 4 or claim 5 wherein the enzyme is a ribonuclease.

7. A method as claimed in claim 4 or claim 5 wherein the enzyme is a deoxyribonuclease.

8. A method as claimed in any one of claims 4 to 7 wherein the enzyme is S1 deoxyribonuclease.

9. A method as claimed in claim 4, claim 5 or claim 7 wherein the enzyme is an exonulcease.

10. A method as claimed in claim 9, wherein the enzyme is T7 exonuclease.

11. A method as claimed in any one of claims 1 to 3 wherein nucleic acid probe that has successfully hybridised is digested by an enzyme that has been chosen to selectively digest at least one strand of double stranded hybridised nucleic acid.

12. A method as claimed in claim 11 wherein the enzyme is a 5' nuclease.

13. A method as claimed in claim 12 wherein the 5' nuclease is also a DNA polymerase.

14. A method as claimed in claim 13 wherein the 5' nuclease/ DNA polymerase is a thermostable enzyme.

15. A method as claimed in claim 14 wherein the thermostable enzyme is *Taq* polymerase.

16. A method as claimed in claim 14 or claim 15 wherein the reaction mixture also comprises a pair of primers suitable for extension by the DNA polymerase.

17. A method as claimed in claim 16 wherein reaction conditions and temperature cycling are suitable for a polymerase chain reaction (PCR) to take place concomitant to the 5' nuclease digestion of probe.

18. A method as claimed in any one of claims 1 to 3, in which a first oligonucleotide probe labelled with an electrochemically active marker is prevented from complete hybridisation by competition from a second oligonucleotide, and the resultant partially hybridised oligonucleotide labelled with an electrochemically active marker is cleaved by an enzyme that specifically recognises the configuration of the two oligonucleotides hybridised onto the target nucleic acid, said cleavage effectively shortening the oligonucleotide portion to which the electrochemically active marker is attached.

19. A method as claimed in any one of claims 1 to 3, in which a first oligonucleotide probe is prevented from complete hybridisation by competition from a second oligonucleotide, and the resultant partially hybridised first oligonucleotide probe is cleaved by an enzyme that specifically recognises the configuration of the two oligonucleotides

hybridised onto the target nucleic acid, the cleavage product being recognised by a recognition cassette which comprises at lease one oligonucleotide and is able to hybridise to the first cleavage product to produce an oligonucleotide configuration recognisable by an enzyme that cleaves a region of the recognition cassette that is labelled with an electrochemically active marker.

20. A method as claimed in any one of the preceding claims for the detection of nucleic acid polymorphisms.

21. A method as claimed in any one of the preceding claims for detection of allelic polymorphisms.

22. A method as claimed in any one of the preceding claims for the detection of single nucleotide polymorphisms.

23. A method as claimed in any one of claims 1 to 19 for the quantification of nucleic acid species.

24. A method as claimed in any one of claims 1 to 19 for the quantification of gene expression.

25. A method as claimed in any one of claims 16 to 24 wherein primer design and/or probe design and/or thermal cycling and detection of electrochemically active marker is carried out automatically or with the assistance of a software-directed microprocessor.

26. A method of detecting a specific protein or group of proteins, comprising: contacting a protein solution with an oligonucleotide probe labelled with an electrochemically active marker, providing conditions at which the probe is able to bind to any specific protein or group of proteins that may be present in the solution, selectively degrading unhybridised nucleic acid probe, and electrochemically determining information relating to the electrochemically active marker in order to provide information about the presence, absence or relative or absolute amounts of the specific target protein or group of target proteins present in said solution.

27. A method as claimed in claim 26 wherein the oligonucleotide probe sequence is substantially similar to an *in vivo* protein recognition site and the protein or group of proteins potentially detected would ordinarily be regarded as a nucleic acid binding protein(s).

28. A method as claimed in claim 26 wherein the oligonucleotide probe comprises an aptamer which has been selected to bind to a specific protein or group of proteins.

29. A method as claimed in any one of claims 24 to 28 wherein the unhybridised nucleic acid is degraded by an enzyme.

30. A method as claimed in claim 29 wherein the enzyme is an endonuclease.

31. A method as claimed in claim 29 or claim 30 wherein the enzyme is a ribonuclease.

32. A method as claimed in any one of claims 29 to 31 wherein the enzyme is a deoxyribonuclease.

33. A method as claimed in any one of claims 29 to 32 wherein the enzyme is S1 deoxyribonuclease.

34. A method as claimed in any one of claims 26 to 33 for the detection of protein polymorphisms.

35. A method as claimed in any one of claims 26 to 34 for the quantification of protein expression.

36. A method as claimed in any one of the preceding claims wherein the electrochemical method is voltammetry.

37. A method as claimed in any one of claims 1 to 35 wherein the electrochemical technique is an amperometric technique.

38. A method as claimed in claim 35 claims wherein the method used is differential pulse voltammetry.

39. A method as claimed in any of the preceding claims wherein the electrochemical technique utilizes one or more electrodes that have been functionally surrounded by a selectively permeable membrane.

40. A method as claimed in claim 39 wherein the membrane is selectively permeable on the basis of molecular size.

41. A method as claimed in claim 39 or claim 40 wherein the membrane is selectively permeable on the basis of charge.

42. A method as claimed in any one of claim 39 to 41 wherein the membrane is selectively permeable on the basis of hydrophobicity or hydrophilicity.

43. Use of a method as claimed in any one of the preceding claims in the detection of a genetic disease or a genetic disease carrier status or a genetic predisposition to disease.

44. Use of a method as claimed in any one of claims 1 to 43 to detect or identify a pathogen in a sample.

45. Use of a method as claimed in any one of claims 1 to 43 to predict a response of an organism to a therapeutic or toxic agent.

46. A nucleic acid probe molecule comprising an oligonucleotide of specific sequence covalently linked to one or more electrochemically active marker moieties.

47. A probe as claimed in claim 46 wherein one or more electrochemically active marker moieties are linked to the oligonucleotide via a linker comprising an aliphatic chain having at least 4 carbon atoms.

48. A probe as claimed in claim 46 or claim 47, which comprises at least one metallocene moiety.

49. A probe as claimed in any one of claims 46 to 48, which comprises at least one ferrocene moiety.

50. A probe as claimed in anyone of claims 46 to 49 wherein the oligonucleotide component is optimised in terms of length or sequence to hybridise to a target nucleic acid sequence.

51. A probe as claimed in any one of claims 46 to 50 wherein the oligonucleotide component is optimised in order to hybridise to a target DNA sequence at a position intermediate between a matched pair of oligonucleotide PCR primers, so that upon primer extension the oligonucleotide component of the probe may be digested by a 5' nuclease activity of the thermostable DNA polymerase.

52. A probe as claimed in any one of claims 46 to 50 wherein the oligonucleotide component is optimised in order to partially hybridise to a target nucleic acid sequence at a position which overlaps with a second hybridised oligonucleotide, the overlap region being situated towards the 5' end of the probe, said 5' end being prevented from complete hybridisation to the target nucleic acid by the presence of the second oligonucleotide.

53. A probe as claimed in any of claims 46 to 52 wherein said probe is a recognition cassette labelled with an electrochemically active marker and optimised to hybridise to a target nucleic acid sequence so as to form a region of nucleic acid triplex which can be specifically recognised by an enzyme, said recognition resulting in cleavage of said recognition cassette.

54. A probe as claimed in any one of claims 46 to 49 wherein the nucleic acid component is optimised in terms of length or sequence to hybridise to a target protein.

55. A probe as claimed in claim 54 wherein the probe comprises an aptamer.

56. A probe claimed in claim 54 or claim 55 wherein the probe substantially comprises the nucleic acid sequence of a naturally occurring protein recognition site.

57. A probe as claimed in any one of claims 46 to 56 wherein an electrochemically active marker is attached to the 3' end of the oligonucleotide probe.

58. A probe as claimed in any one of claims 46 to 57 wherein an electrochemically active marker is attached to the 5' end of the oligonucleotide probe.

59. A probe as claimed in any one of claims 46 to 58 wherein multiple electrochemically active markers are attached along the length of the oligonucleotide probe.

60. A probe as claimed in any one of claims 46 to 59 wherein an electrochemically active marker is attached to substantially all of nucleotide residues of the oligonucleotide probe.

61. A probe as claimed in any one of claims 46 to 60 wherein one of more electrochemically active marker moiety is as according to formula I, II, III, IV, VII or VIII.

62. An oligonucleotide probe substantially as described in any of the examples herein.

63. A probe as claimed in any one of claims 46 to 62 wherein the oligonucleotide component is phosphorylated at both the 3' and 5' ends.

64. A kit comprising an oligonucleotide labelled with an electrochemically active marker and any one or more other component selected from oligonucleotide primers or enzymes optimised for use with the labelled oligonucleotide in accordance with any of the preceding method or use claims.

65. A kit as claimed in claim 64, comprising an oligonucleotide probe labelled with an electrochemically active marker and S1 nuclease.

66. A kit as claimed in claim 64, comprising an oligonucleotide probe and a pair of PCR primers.

67. A kit as claimed in claim 64 or claim 66, comprising a nucleic acid polymerase that exhibits a 5' nuclease activity.

68. Apparatus arranged to carry out any one or more of method claims 1 to 35, 39 to 45, 91 or 92.

69. Apparatus comprising one or more sample receiving regions for receiving one or more sample means for controlling the temperature of said sample receiving regions and means for measuring the electrochemical properties of said samples.

70. Apparatus as claimed in claim 69, wherein the apparatus comprises a thermal cycler.

71. Apparatus as claimed in claim 69 or 70, wherein comprises apparatus for voltammetry.

72. A container comprising one or more sample receiving regions for holding one or more samples for use in the apparatus as claimed in any of claims 69 to 71.

73. A container as claimed in claim 73 adapted to receive at least one electrode component for measuring the electrochemical properties of said samples.

74. A container as claimed in claim 72 or claim 73 wherein the container comprises at least one integral electrode component.

75. A compound of formula XI,

$$Mc\text{-}NR'\text{-}C(=O)\text{-}X\text{-}(Ar)_n\text{-}(L)_m\text{-}R \qquad XI$$

Wherein

- Mc is a metallocenyl group in which each ring may independently be substituted or unsubstituted,
- the metallocenyl group comprises a metal ion M selected from the group consisting of iron, chromium, cobalt, osmium, ruthenium, nickel or titanium,
- R' is H or lower alkyl,
- X is either NR' or O,
- Ar is a substituted or unsubstituted aryl group,
- n is 0 or 1,

- L is a linker group,
- m is 0 or 1, and
- R represents a moiety to be labelled or R is a moiety comprising a leaving group.

76. A compound as claimed in claim 75 in which the Mc group is substituted by one or more groups selected lower alkyl (for example C_1 to C_4 alkyl), lower alkyl substituted with a hydroxy, halo, cyano, oxo, amino, ester or amido group, lower alkenyl, lower alkenyl substituted with a hydroxy, halo, cyano, oxo, amino, ester or amido group, aryl or aryl substituted with a hydroxy, halo, cyano, oxo, amino, ester or amido group.

77. A compound as claimed in claim 75 in which the Mc group is unsubstituted.

78. A compound as claimed in any one of claims 75 to 77 in which M is an iron ion.

79. A compound as claimed in any one of claims 75 to 78 in which R' is H.

80. A compound as claimed in any one of claims 75 to 79 in which X is NH.

81. A compound as claimed in any one of claims 75 to 80 in which n=1.

82. A compound as claimed in any one of claims 75 to 80 in which n=0.

83. A compound as claimed in any one of claims 75 to 82 in which m=1.

84. A compound as claimed in any one of claims 75 to 82 in which m=0.

85. A compound as claimed in any one of claims 75 to 84 in which R is a moiety to be labelled and R comprises amino acid, nucleotide, nucleoside, sugar, peptide, protein, oligonucleotide, polynucleotide, carbohydrate or derivative of any thereof.

86. A compound as claimed in any one of claims 75 to 84 in which R is a group comprising a leaving group.

87. A compound as claimed in claim 86 wherein R is a group comprising *N*-hydroxysuccinimide.

88. A compound as claimed in any one of claims 75 to 85 wherein R comprises an oligonucleotide having a sequence that enables it to hybridize with a target.

89. A compound as claimed in any of claims 75 to 88, wherein the compound is electrochemically active or becomes electrochemically active following partial cleavage.

90. A compound as claimed in any of claims 75 to 89, wherein the metallocene group is substituted by any other electrochemically active marker group.

91. A method as claimed in any one of claims 1 to 42 in which two or more oligonucleotide probes are used, each probe being labelled with a different electrochemically active marker.

92. A method as claimed in claim 91 in which the two or more electrochemically active markers have peaks in their voltammogram traces that are resolvable from each other.